

882
9/10/03

~~WE CLAIM~~ CLAIMS
WHAT IS CLAIMED IS:

1. An osteoclastogenesis inhibitory factor protein comprising the following properties:
 - (a) molecular weights as determined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of approximately 60 kD under reducing conditions, and approximately 60 kD and 120 kD under non-reducing conditions;
 - (b) high affinity to cation-exchange resins and heparin derivatized substrates;
 - (c) inhibition activity: inhibits osteoclast differentiation or maturation, wherein the inhibition activity is decreased by heating at about 70°C for about 10 min. or at about 56°C for about 30 min., and wherein said activity is lost by heating at about 90°C for about 10 min.; and
 - (d) internal amino acid sequences substantially in accordance with Seq. ID Nos. 1, 2 and 3.
2. The protein of claim 1 comprising the N-terminal amino acid sequences provided in Seq. ID No. 7.
3. The protein of claim 1 which is derived from human fibroblasts.
4. A method of producing an osteoclastogenesis inhibitory factor protein comprising the steps of
 - cultivating human fibroblast cells;
 - forming a lysate of said fibroblast cells; and
 - separating said factor from said fibroblast cell lysate by a combination of ion-exchange, affinity, and reverse phase chromatography.
5. The method of claim 4 further comprising the step of cultivating the human fibroblasts on alumina ceramic pieces.

6. A protein comprising an amino acid sequence as provided in Seq. ID No. 4.
7. cDNAs encoding an amino acid sequence as provided in Seq. ID No. 4.
8. cDNA comprising a nucleotide sequence as provided in Seq. ID No. 6.
9. cDNAs that hybridize to a cDNA as provided in Seq. ID No. 6 under moderately stringent conditions.
10. A protein expressed from cDNA encoding an amino acid sequence as provided in Seq. ID No. 4.
11. A protein having a biological activity to inhibit osteoclast differentiation or maturation, said protein having an amino acid sequence expressed from a cDNA sharing at least about 80% sequence identity with the amino acid sequence provided in Seq. ID No. 4.
12. A recombinant protein which inhibits osteoclast differentiation or maturation expressed from a cDNA encoding an amino acid sequence as provided in Seq. ID No. 4; said protein comprising the following properties:
- (a) molecular weights as determined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of approximately 60 kD under reducing conditions, and approximately 60 kD and 120 kD under non-reducing conditions;
 - (b) high affinity to cation-exchange resins and heparin derivatized substrates;
 - (c) inhibitory activity: inhibits osteoclast differentiation or maturation, wherein said activity is decreased by heating at about 70°C for about 10 min. or at about 56°C for about 30 min., and wherein said activity is lost by heating at about 90°C for about 10 min.; and
 - (d) an internal amino acid sequence as provided in Seq. ID Nos. 1-3.

13. The protein of claim 10 produced by gene engineering using mammalian cells as host cells.
14. The protein of claim 13 wherein said mammalian cells are 293/EBNA cells or CHO cells.
15. A cDNA comprising a nucleotide sequence as provided in Seq. ID No. 8.
16. A protein encoded by a cDNA having a nucleotide sequence as provided in Seq. ID No. 8.
17. cDNAs encoding amino acid sequence as provided in Seq. ID No. 9.
18. A cDNA comprising a nucleotide sequence as provided in Seq. ID No. 10.
19. A protein encoded by a cDNA comprising a nucleotide sequence as provided in Seq. ID No. 10.
20. cDNAs encoding an amino acid sequence as provided in Seq. ID No. 11.
21. A cDNA comprising a nucleotide sequence as provided in Seq. ID No. 12.
22. A protein encoded by a cDNA having a nucleotide sequence as provided in Seq. ID No. 12.
23. cDNAs encoding an amino acid sequence as provided in Seq. ID No. 13.
24. A cDNA comprising a nucleotide sequence as provided in Seq. ID No. 14.
25. A protein encoded by a cDNA having a nucleotide sequence as provided in Seq. ID No. 14.

26. cDNAs encoding an amino acid sequence as provided in Seq. ID No. 15.
27. An antibody having specific affinity to the osteoclastogenesis inhibitory factor protein of claim 1.
28. The antibody of claim 27 that is polyclonal.
29. The antibody of claim 27 that is monoclonal.
30. The monoclonal antibody of claim 29 comprising the following properties: a molecular weight of about 150,000, and of subclass IgG₁, IgG_{2a}, or IgG_{2b}.
31. A method for determining the concentration of an osteoclastogenesis inhibitory factor protein comprising contacting a sample suspected of containing said protein with an antibody of claim 27, 28, 29 or 30 under conditions sufficient to induce formation of protein-antibody conjugates, and detecting the amount of protein-antibody conjugates formed.

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